

Chromosome Counts for the Asian Myristicaceae (Magnoliales)

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Myristicaceae, a family with pantropical distribution and sister to the rest of the Magnoliales, are poorly understood with respect to chromosome numbers. Here we report somatic chromosome numbers of 14 species in five of the six Asian genera. Chromosome numbers were consistent within individual genera but markedly differ among the genera. Based on our observations, we concluded that the generic base number is $x = 20$ in *Endocomia*, $x = 22$ in *Gymnacranthera* and *Knema*, $x = 25$ in *Myristica*, and $x = 26$ in *Horsfieldia*. Although reliable data on chromosome numbers are currently limited to Asian genera, a great divergence in base numbers is a marked contrast to the low molecular divergence within the family, casting a doubt to dating Myristicaceae to be young.

Key words: chromosome number, Magnoliales, Myristicaceae

Myristicaceae comprise around 400 species of trees in about 20 genera more or less equally distributed in the tropics worldwide: eight genera in Africa, six genera in America, and six genera in Southeast Asia (de Wilde 2000, see Table 1). Molecular analyses show that Myristicaceae are placed in Magnoliales and are sister to the rest of the order (Sauquet *et al.* 2003, Stevens 2001 onwards). Several morphological characters, such as androecium and pollen morphology, were recently investigated in Myristicaceae (Sauquet 2003, Sauquet & Le Thomas 2003), but many other morphological characters are yet poorly understood. In particular, chromosome numbers which often reflect evolutionary relationships among families (e.g., Oginuma & Tobe 2006: Laurales), or within families (e.g., Oginuma & Tobe 1997: Rhizophoraceae), have been poorly studied in the family (for review see Kühn & Kubitzki 1993). Chromosome counts published so far are restricted to eight genera including four Southeast Asian genera (*Gymnacranthera* Warb., *Horsfieldia* Willd., *Knema* Lour. and *Myristica*

Gronov.). Twelve (60%) of the 20 genera remain chromosomally unknown (see Table 1). Even those reported are inconsistent, as in case of *Myristica* (see Table 1).

In this paper we report the results of an investigation of somatic chromosome numbers of 14 species of five Southeast Asian genera (*Endocomia* W.J. de Wilde, *Gymnacranthera*, *Horsfieldia*, *Knema*, and *Myristica*) in Myristicaceae, and provide a brief note on chromosome counts previously reported. The chromosome counts, which we present for all the Asian genera except *Paramyristica* W. J. de Wilde (*P. sepicana* (Foreman) W. J. de Wilde only), which is endemic to Papua New Guinea, provide a better understanding of chromosome features of the family.

Materials and Methods

The 14 species examined are listed in Table 2 along with their respective collection data and chromosome numbers. Somatic chromosomes were examined using young leaves collected

TABLE 1. Summary of chromosome information on Myristicaceae and generic base number. Chromosome numbers reported in present study are indicated in bold.

Taxon	Distribution	Chromosome numbers	Base number
<i>Brochoneura</i> Warb.	Africa	?	
<i>Cephalosphaera</i> Warb.	Africa	?	
<i>Coelocaryon</i> Warb.	Africa	?	
<i>Compsonura</i> (DC.) Warb.	America	$2n = \text{ca. } 102^{1)}$	
<i>Dialyanthera</i> Warb.	America	?	
<i>Endocomia</i>	Asia	$2n = \mathbf{40}$	$x = 20$
<i>Gymnacranthera</i>	Asia	$n = 21$ $2n = \mathbf{44}$	$x = 22$
<i>Haematodendron</i> Capuron	Africa	?	
<i>Horsfieldia</i>	Asia	$n = 25$ $2n = 50, \mathbf{52}$	$x = 26$
<i>Iryanthera</i> Warb.	America	$2n = 100^{2)}$	
<i>Knema</i>	Asia	$n = 21$ $2n = \mathbf{44}$	$x = 22$
<i>Mauloutchia</i> Warb.	Africa	?	
<i>Myristica</i>	Asia	$n = 19, 21, 26$ $2n = \mathbf{50}$	$x = 25$
<i>Osteophloeum</i> Warb.	America	$2n = \text{ca. } 280^{3)}$	
<i>Otoba</i> (DC.) H.Karst.	America	?	
<i>Paramyristica</i>	Asia	?	
<i>Pycnanthus</i> Warb.	Africa	$2n = 38^{4)}$	
<i>Scyphocephalum</i> Warb.	Africa	?	
<i>Staudtia</i> Warb.	Africa	?	
<i>Viola</i> Aubl.	America	$2n = 52^{5)}$	

References for previous chromosome counts for species of *Gymnacranthera*, *Horsfieldia*, *Knema*, and *Myristica* are presented in text.

¹⁾ Data from *Compsonura ulei* Warb. (Morawetz 1986).

²⁾ Data from *Iryanthera coriacea* Ducke (Morawetz 1986).

³⁾ Data from *Osteophloeum platyspermum* Warb. (Morawetz 1986).

⁴⁾ Data from *Pycnanthus angolensis* (Welw.) Warb. (Mangenot & Mangenot 1957).

⁵⁾ Data from *Viola calophylla* Warb., *V. divergens* Ducke, and *V. elongata* Warb. (Morawetz 1986).

from trees in the field. Methods of pretreatment, fixation, and staining for chromosomes followed Oginuma *et al.* (1992). At least three to five cells were examined to determine the chromosome number.

Terminology of chromosome morphology on the basis of the position of the centromere follows Levan *et al.* (1964).

Results and Discussion

Chromosome counts of individual genera

Endocomia

The genus *Endocomia* comprises four species distributed from southern China to New Guinea (de Wilde 2000). We observed chromosomes for the first time for the genus using *E. canarioides*

(King) W.J. de Wilde. The species had $2n = 40$ (Figs. 1, 2). The chromosomes at metaphase gradually varied in length from 0.7 to 1.7 μm . Of the 40 chromosomes, some had a centromere in a median position, but the centromere position in others was uncertain. The base number of this genus is $x = 20$.

Gymnacranthera

The genus *Gymnacranthera* comprises seven species distributed from southern Peninsular Thailand to New Guinea (de Wilde 2000). We studied two species *G. farquhariana* Warb. var. *farquhariana* (Figs. 3, 4), and *G. forbesii* Warb. (Figs. 5, 6), both of which had $2n = 44$. The chromosomes at metaphase gradually varied in length from 0.3 to 0.7 μm . In both species, many of the

TABLE 2. Taxa of Myristicaceae studied, voucher information and chromosome numbers.

Taxon	Collection	Chromosome number
<i>Endocomia canarioides</i>	SINGAPORE. Bukit Timah <i>Oginuma & Lum 0701</i> (KYO)	$2n = 40$
<i>Gymnacranthera farquhariana</i> var. <i>farquhariana</i>	SINGAPORE. Bukit Timah <i>Oginuma & Lum 0702</i> (KYO)	$2n = 44$
<i>G. forbesii</i>	SINGAPORE. MacRitchie <i>Oginuma & Lum 9703</i> (KYO)	$2n = 44$
<i>Horsfieldia irya</i>	Cultivated, Singapore Botanical Garden. <i>Oginuma & Lum 0704</i> (KYO)	$2n = 52$
<i>H. sucosa</i>	SINGAPORE. MacRitchie <i>Oginuma & Lum 0705</i> (KYO)	$2n = 52$
<i>H. superba</i>	Cultivated, Singapore Botanical Garden. <i>Oginuma & Lum 0706</i> (KYO)	$2n = 52$
<i>H. wallichii</i>	SINGAPORE. Bukit Timah <i>Oginuma & Lum 0707</i> (KYO)	$2n = 52$
<i>Knema furfuracea</i>	SINGAPORE. Bukit Timah <i>Oginuma & Lum 0708</i> (KYO)	$2n = 44$
<i>K. globularia</i>	SINGAPORE. Ubin Is. <i>Oginuma & Lum 0709</i> (KYO)	$2n = 44$
<i>K. latericia</i>	Cultivated, Singapore Botanical Garden. <i>Oginuma & Lum 0710</i> (KYO)	$2n = 44$
<i>K. laurina</i>	Cultivated, Singapore Botanical Garden. <i>Oginuma & Lum 0711</i> (KYO)	$2n = 44$
<i>Myristica cinnamomea</i>	SINGAPORE. MacRitchie. <i>Oginuma & Lum 0712</i> (KYO)	$2n = 50$
<i>M. elliptica</i>	Cultivated, Singapore Botanical Garden. <i>Oginuma & Lum 0713</i> (KYO)	$2n = 50$
<i>M. fragrans</i>	Cultivated, Singapore Botanical Garden. <i>Oginuma & Lum 0714</i> (KYO)	$2n = 50$

44 chromosomes had a centromere in a median position, but the centromere position in others was uncertain.

There are three previous records on chromosome counts of *Gymnacranthera*: $n = 21$ from *G. forbesii* (Kühn & Kubitzki 1993) and *G. paniculata* Warb. (Ehrendorfer *et al.* 1968), and $2n = 44$ from *G. paniculata* (Oginuma *et al.* 1999) (Table 1). The chromosome number of $2n = 44$ from the three species examined (i.e., *G. farquhariana*, *G. forbesii*, and *G. paniculata*) indicates that the base number of *Gymnacranthera* is $x = 22$. The chromosome count $n = 21$ reported for *G. forbesii* and *G. paniculata* appears to be erroneous, at least requiring confirmation.

Horsfieldia

The genus *Horsfieldia* comprises about 100 species that occur from Sri Lanka and southern China east to the Solomon Islands and northern Australia (de Wilde 2000). We studied four species *H. irya* Warb., *H. sucosa* Warb. (Figs. 7, 8), *H. superba* Warb. (Figs. 9, 10), and *H. wallichii* Warb. (Figs. 11, 12), which all had $2n = 52$. Chromosomes at metaphase of the three species gradually varied in length from 0.3 to 1.0 μm . Of the 52 chromosomes, many had a centromere in a median position, but the centromere position in others was uncertain.

The previous chromosome counts for *Horsfieldia* are $n = 25$ (Ehrendorfer *et al.* 1968) and $2n = 50$ (Morawetz 1986) for *H. odorata* Willd. The four species examined in our study (i.e., *H. irya*,

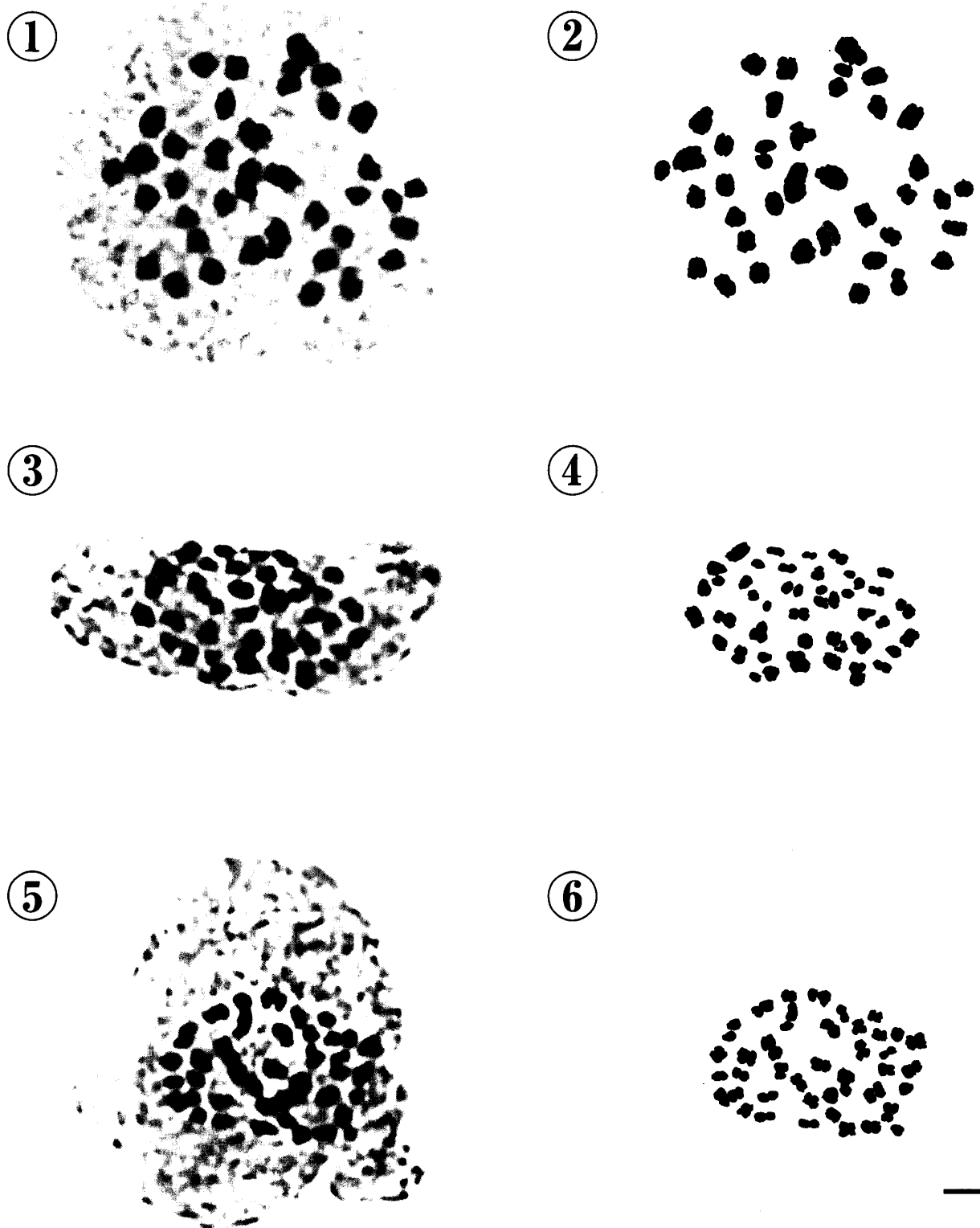


FIG. 1–6. Somatic chromosomes at mitotic metaphase in Myristicaceae. 1, 3, 5: micrographs. 2, 4, 6: drawings of micrographs in 1, 3, and 5, respectively. 1, 2: *Endocomia canarioides* ($2n = 40$). 3, 4: *Gymnacranthera farquhariana* var. *farquhariana* ($2n = 44$). 5, 6: *G. forbesii* ($2n = 44$). Scale bar = 2 μm .

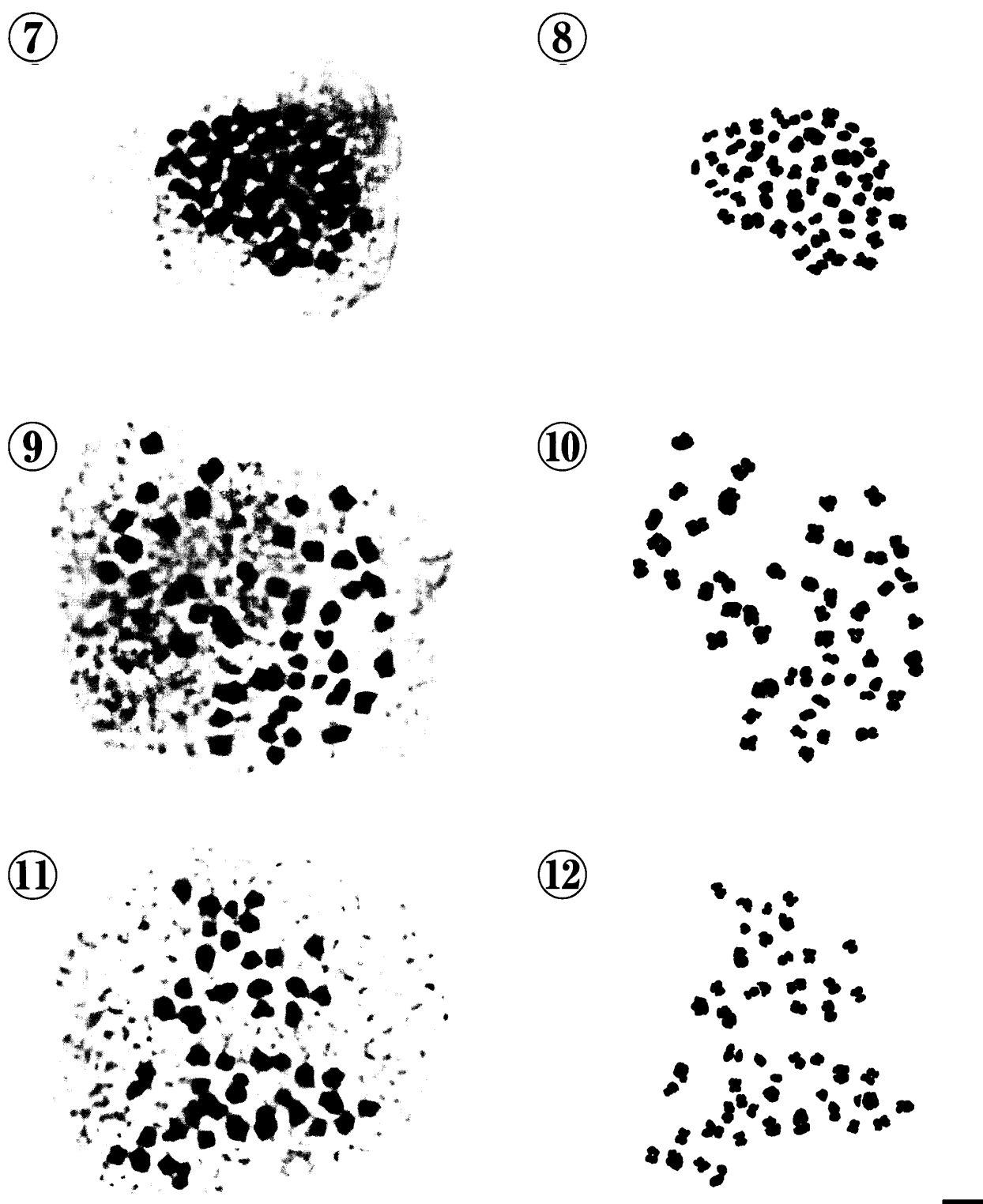


FIG. 7–12. Somatic chromosomes at mitotic metaphase in Myristicaceae. 7, 9, 11: micrographs. 8, 10, 12: drawings of micrographs in 7, 9, and 11, respectively. 7, 8: *Horsfieldia sucosa* ($2n = 52$). 9, 10: *H. superba* ($2n = 52$). 11, 12: *H. wallichii* ($2n = 52$). Scale bar = 2 μm .

H. sukosa, *H. superba*, and *H. wallichii*) had $2n = 52$, indicating a base number of $x = 26$ in *Horsfieldia*. The previous chromosome counts of $n = 25$ and $2n = 50$ for *H. odorata* appears to be incorrect and require confirmation.

Knema

The genus *Knema* comprises about 90 species that occur from India to western New Guinea (de Wilde 2000). We studied four species of *Knema*: *K. furfuracea* Warb., *K. globularia* Warb. (Figs. 13, 14), *K. latericia* Elmer (Figs. 15, 16), and *K. laurina* Warb. (Figs. 17, 18), which all had $2n = 44$. Chromosomes at metaphase of the four species gradually varied in length from 0.3 to 1.2 μm . Some of the 44 chromosomes had a centromere in a median, submedian, or subterminal position, but the centromere position in others was uncertain.

Ehrendorfer *et al.* (1968) previously reported a count for *K. intermedia* (Blume) Warb. of $n = 21$. All four species examined in our study (*K. furfuracea*, *K. globularia*, *K. latericia*, and *K. laurina*) had $2n = 44$, indicating that the base number of *Knema* is $x = 22$. The chromosome count $n = 21$ of *K. intermedia* requires confirmation.

Myristica

The genus *Myristica* comprises about 170 species that occur from India to northern Australia and far into the Pacific (de Wilde 2000). We studied three species of *Myristica*: *M. cinnamomea* King (Figs. 19, 20), *M. elliptica* Wall. (Figs. 21, 22), and *M. fragrans* Houtt. (Figs. 23, 24). All had $2n = 50$. Chromosomes at metaphase gradually varied in length from 0.3 to 1.2 μm . In all the three species examined, some of the 50 chromosomes had a centromere in a median, submedian, or subterminal position, but the centromere position in others was uncertain.

Various chromosome numbers have been recorded previously: $n = 19$ in *M. insipida* R. Br. (Soltis & Soltis 1990), $n = 21$ in *M. elliptica* (Ehrendorfer *et al.* 1968) and *M. fragrans* (Simmonds 1954), $n = 26$ in *M. erratica* Hook. f. & Thompson (Mehra & Bawa 1969), and $2n = 50$ from *M.*

zeylanica Thwaites (Morawetz 1986). Our counts of three species agreed only with Morawetz (1986). Chromosome numbers may vary within the genus, but our results indicate that the base number of *Myristica* is $x = 25$.

Chromosome evolution in Myristicaceae

In the present study we determined the chromosome base number for the five Asian genera of Myristicaceae: $x = 20$ in *Endocomia*, $x = 22$ in *Gymnacranthera* and *Knema*, $x = 25$ in *Myristica*, and $x = 26$ in *Horsfieldia*. It is premature to discuss chromosome evolution within the family because exact chromosome information on Myristicaceae is restricted to these five Asian genera. Of interest, however, is the fact that the Laurales, which are considered to be sister to the Magnoliales (e.g., Soltis *et al.* 2011), have $x = 11$ as a probable archaic base number (Oginuma & Tobe 2006). If $x = 11$ is plesiomorphic in Magnoliales as well, the base number $x = 22$ (in *Gymnacranthera* and *Knema*) might have been derived by polyploidization from $x = 11$. The other numbers $x = 20$, 25, and 26 may have been derived by an aneuploid decrease and increase from $x = 22$.

Compared to other families of the Magnoliales, Annonaceae ($x = 7, 8, 9$) and Magnoliaceae ($x = 19$) (Okada 1975), Myristicaceae have a great diversity in generic base numbers, with at least $x = 20, 22, 25, 26$. In contrast, Doyle *et al.* (2004) showed strikingly low molecular divergence, based on several plastid analyses, in the Myristicaceae. An estimated age by molecular dating for the crown-group of the family was 15–18 or 17–21 Myr, suggesting either a young age for the family or a marked slowdown in molecular evolution (Doyle *et al.* 2004). Doyle *et al.* (2004) further discuss that the oldest diagnostic fossils of the Myristicaceae are Miocene seeds which can be taken as evidence for the young age of the family, but that is implausible for the transoceanic dispersal of the large, animal-dispersed seeds in the family. Although exact chromosome data are currently limited to the Asian taxa, a great diversity in their base numbers casts doubt on a young date for Myristicaceae, but instead suggests that the family is much older. Further

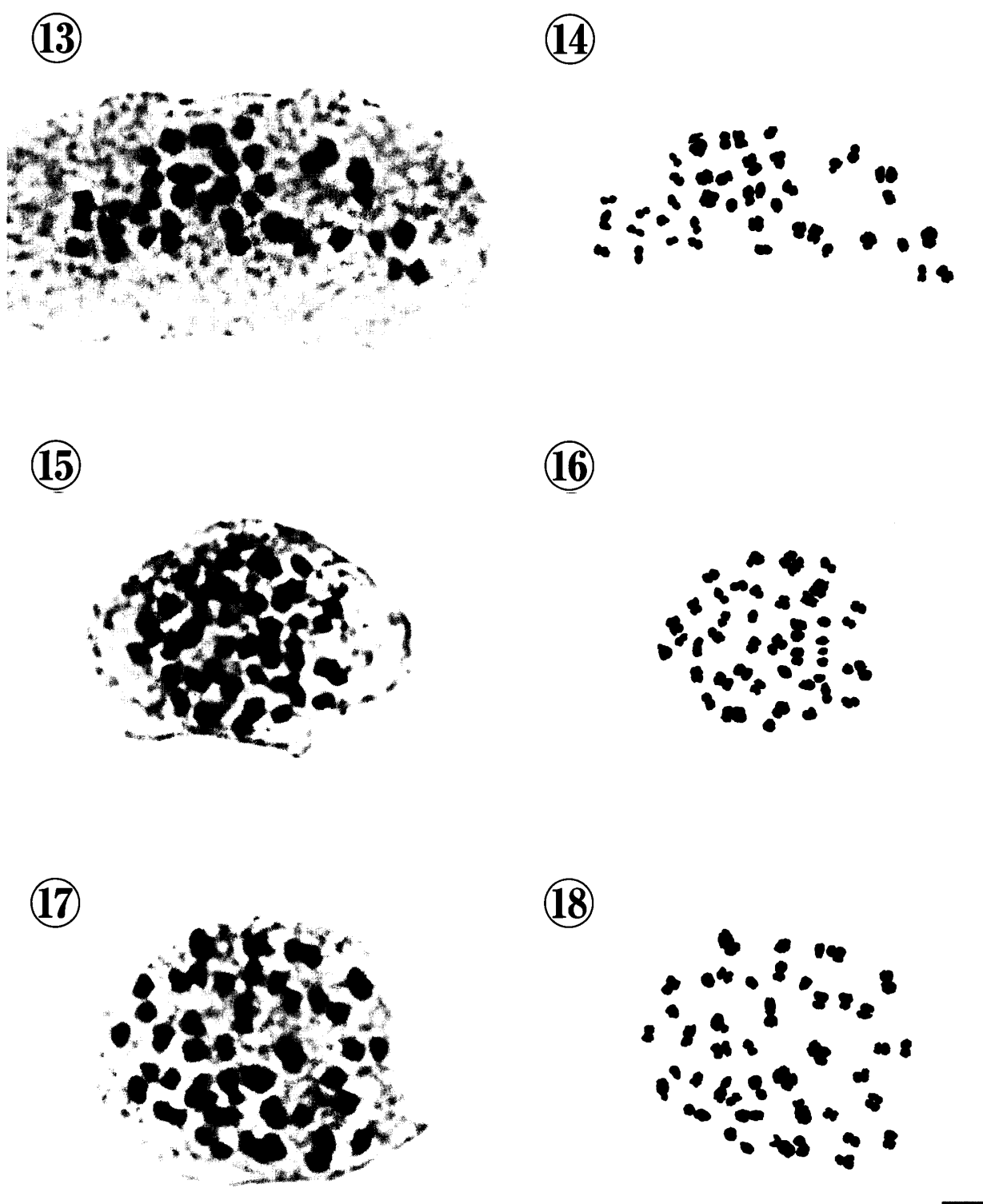
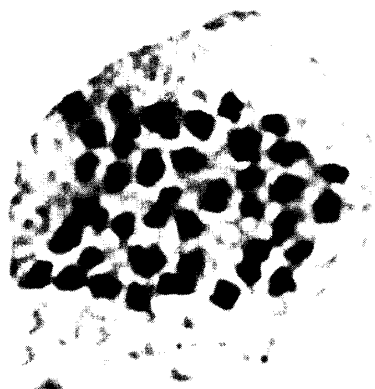
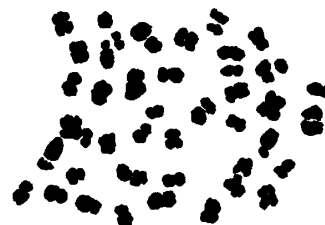


FIG. 13–18. Somatic chromosomes at mitotic metaphase in Myristicaceae. 13, 15, 17: micrographs. 14, 16, 18: drawings of micrographs in 13, 15, and 17, respectively. 13, 14: *Knema globularia* ($2n = 44$). 15, 16: *K. latericia* ($2n = 44$). 17, 18: *K. laurina* ($2n = 44$). Scale bar = 2 μm .

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20



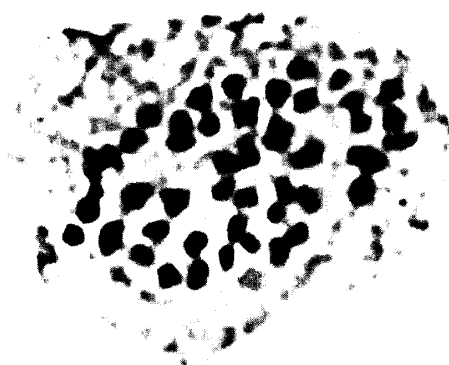
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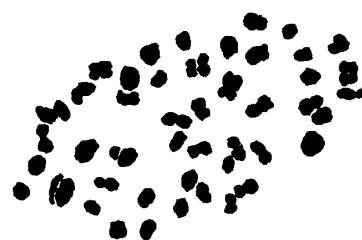


FIG. 19–24. Somatic chromosomes at mitotic metaphase in Myristicaceae. 19, 21, 23: micrographs. 20, 22, 24: drawings of micrographs in 19, 21, and 23, respectively. 19, 20: *Myristica cinamonea* ($2n = 50$). 21, 22: *M. elliptica* ($2n = 50$). 23, 24: *M. fragrans* ($2n = 50$). Scale bar = 2 μm .

studies are needed of chromosome numbers in the African and American taxa.

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